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Phylogenetic Studies on Some Wild *Brassica* Species

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Summary

Investigation of mode of chromosome conjugation at MI in the microsporogenesis in some species and genus hybrids including their induced tetraploids was made in order to make clear the phylogenetic relationship of the genomes of *Brassica adpressa* (A_d, n=7), *B. fruticulosa* (*F*, n=8) and *B. Tournefortii* (*T*, n=10) to the known genomes of *B. campestris* (*A*, n=10), *B. oleracea* (*C*, n=9), *B. nigra* (*B*, n=8), *Sinapis arvensis* (S_{ar}, n=9) and *Raphanus sativus* (*R*, n=9). The occurrence of an indefinite number of bivalents in a hybrid and of quadrivalents in the corresponding tetraploid hybrid were always observed, in most cases the maximum number of the former coinciding with that of the latter. The result of the examination of the origin of the bivalents formed in the diploid hybrids showed that all or a part of them were the product of allosyndesis between the two different genomes being concurrent in the hybrid nucleus and the obvious partially homologous relation was confirmed between A_d—*A*, *F*—*A*, *F*—*B*, *T*—*A*, *T*—*C*, *T*—S_{ar}, and *T*—*R*. The degree of affinity between the genomes represented in terms of the number of allosyndetic bivalents was not always higher within *Brassica* than between *Brassica* and other genera. From the results obtained in the present experiment as well as those previously reported it was pointed out that the intricate inter-relationship among the genomes of *Brassica*, *Sinapis*, *Eruca* and *Raphanus* which were proved already by the author to be of an aneuploid nature, having been derived from a common original genome, made it difficult to fix generic boundaries among them from the phylogenetic standpoint.

Citing actual illustrations, discussion concerning the utilization of synthetic plants in *Brassiceae* was made, which resulted in the conclusion that they could be important materials for introducing necessary genes from one genome to the other, for making an alien substitution race, and for obtaining an alloplasmatic strain, even if they were not worth cultivating as such.

As far as the author is aware, a phylogenetic investigation of the genomes of the 3 wild species of *Brassica*, *i.e.*, *adpressa* (n=7), *fruticulosa* (n=8) and *Tournefortii* (n=10), has never been made by any worker, save the affirmation of a partially homologous relation between those of *B. Tournefortii* and *B. campestris* (n=10) re-

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ported by Fukushima and Iwasa (1). The author and his collaborators (2) made a plant exploration in the Mediterranean, the Near Eastern and the Middle Eastern regions in the spring of 1965 and collected many strains of wild species belonging to the tribe *Brassiceae* including those of the above-mentioned 3 species. In this paper he intends to report the results of the hybridization experiment with the newly introduced strains of *B. adpressa* and *B. fruticulosa* together with some unpublished data obtained before 1965, using a strain of *B. Tournefortii* from Scotland which was grown hitherto in Sendai.

Materials and Methods

The species used are shown in Table 1 with their origin, haploid number of chromosomes and genome symbol. The symbols representing the two new genomes of *B. adpressa* and *B. fruticulosa* have been designated by the author as A_d and F respectively.

The 3 wild *Brassica* species referred to above have never been used in the author's hybridization experiments and thus a biref description of them is given below.

TABLE 1. *Species Used*

Species	Genome symbol and haploid number of chromosmes	Strain	Origin
Brassica adpressa Boiss.	A_d (n=7)	Ad102	Collected in Carthage, Tunisia
B. fruticulosa Cyril.	F(n=8)	Fr103	Collected near Naples, Italy
〃	〃	Fr104	From Fac. of Pharmacy, Ciudad Univ., Madrid, Spain
B. Tournefortii Gouan	T(n=10)	T156	From Scott. Pt. Breed. Sta., Roslin, Scotland
B. campestris L. var. *chinensis*	A(n=10)	C333	From Watanabe Seed Co., Kogota, Japan
〃	〃	C334	〃
var. *sarson* (=*B. trilocularis* Roxb.)	$A(A')$ (n=10)	C632	From Swedish Seed Assoc., Svalöf, Sweden
B. oleracea L.	C(n=9)	O4	From Watanabe Seed Co., Kogota, Japan
B. nigra Koch	B(n=8)	Ni101	From Nat. Agr. Exp. Sta., Konosu, Japan
Sinapis arvensis L.	S_{ar}(n=9)	Ar102	From Swedish Seed Assoc., Svalöf, Sweden
Raphanus sativus L.	R(n=9)	R105	From Watanabe Seed Co., Kogota, Iapan

B. adpressa Boiss. whose synonyms are *Hirschfeldia incana* Lagr., *H. adpressa* Moench and *Sinapis incana* L. is quite popular in the Mediterranean region and is noticed to have the lowest number of chromosomes of all the species in *Brassica*.

It has rosetted, long-petioled, elliptical leaves with irregular incisions in the lower part and the same-shaped, short-petioled upper leaves which are dark green, more or less coriaceous and pubescent. It bears tiny flowers with yellow petals 4–7 mm long. Its silique is 15–20 mm in length with peculiar knob-shaped beak, which is adpressed to the stem.

The number of chromosomes of *B. fruticulosa* Cyril. seems not to have been reported by any worker, which has been confirmed by the author to be n=8. It seems to be distributed mostly in the coastal region of southern Europe, being especially abundant on the Tyrrhenian coast of Italy. The two strains used are remarkably different from each other in morphology. Fr103 is more or less hispid. Its rosette leaves resemble those of *B. nigra* in shape, but are easily discriminated from the latter by being darker green and coriaceous. Fr104 is a glabrous plant with rosetted, oblong leaves with irregular incisions which are long-petioled, dark green and coriaceous. Both the strains bear yellow flowers whose ovaries develop into siliqua far larger in size than those of *B. nigra*.

B. Tournefortii Gouan is distributed widely throughout the Mediterranean and the Near and the Middle Eastern regions. It is remarkably drought resistant and grows preferably in sandy soils. It is mostly a hispid plant with rosetted, lyrate-cleft leaves and lanceolate, sessile upper leaves. Its indefinite inflorescence ceases to develop soon, leaving only a small number of functional flower buds. Its corolla has narrow, pale yellow petals which do not extend and stigma receives pollens from introrse anthers as pistil develops. It is self-compatible.

The strain C632 is *B. campestris* var. *sarson* whose synonym is *B. trilocularis* Roxb., which is an Indian rape variety called *'yellow sarson'*. Because of its clear-cut difference in morphology from other *campestris* forms, the self-fertile reproductivness, and a marked tendency to decreased fertility in hybrids between it and other forms, Olsson (3) regarded it as a well differentiated subspecies of *campestris*. Fukushima and Iwasa (1), chiefly from the mode of chromosome pairing $9_{II}+2_{I}$ instead of 10_{II} observed, though quite rarely, at MI in the microsporogenesis in their F_1 *trilocularis* × *campestris*, have concluded that the genome of *trilocularis* is slightly different from the *A* genome of *campestris* and have given the designation *A'* to the former.

Corssings were made exclusively by means of bud pollination, using potted plants grown under glass-house conditions. Karyological investigation was made on temporary smear preparations stained with acetocarmine.

Results

1. The result of crossings.

A number of crossings were made among the species used, but, for simplicity's sake, only those which showed the maximum cross ability in the respective species combinations are given in Table 2. More than 3 plants of each species

were pollinated with pollens of the same plant of other species to be crossed. The result was different with different female plants, showing their obvious difference in cross affinity to the same pollens. A certain plant gave relatively abundant true hybrid seeds which were remarkably smaller in size than its normal seeds, while others gave only a very few or no such seeds and some of normal-sized which gave rise to exclusively matromorphic false hybrids. The circumstances here were quite the same as those observed in the case of the species and the genus crosses previously reported (4).

Because of lack of dominance in such traits as shape, color, coriaceousness and pubescence of rosetted and upper leaves, size and shape of petals, degree of extension of calyces, shape of siliqua, and growth habit, the F_1 hybrids obtained showed apparently intermediate morphology between their respective parental species. Occurrence of dominance was, however, noticed in the following characters.

Leaves: petioled>sessile, not clasping≷clasping.**

Corolla: extrorse(anther)>introrse, yellow(petal)>pale yellow.

Inflorescence: developing>not fully developing.

TABLE 2. *Results of Crosses.*

Cross combination	Number of flowers pollinated (A)	Number of hybrids obtained (B)	Cross ability (B)/(A)	Symbol of F_1
B. campestris (C334) ♀ ×*B. adpressa* (Ad102) ♂	62	35	0.56	$CAdF_1I$
B. campestris (C334) ♀ ×*B. fruticulosa* (Fr104) ♂	198	34	0.17	$CFrF_1I$
B. campestris (C333) ♀ ×*B. fruticulosa* (Fr103) ♂	42	8	0.19	$CFrF_1II$
B. fruticulosa (Fr104) ♀ ×*B. campestris* (C334) ♂	97	16	0.17	$FrCF_1I$
B. fruticulosa (Fr104) ♀ ×*B. campestris* (C333) ♂	139	(407)*	(2.93)*	$FrCF_1II$
B. fruticulosa (Fr104) ♀ ×*B. nigra* (Ni101) ♂	133	12	0.09	$FrNiF_1I$
B. Tournefortii (T161) ♀ ×*B. oleracea* (O4) ♂	24	4	0.17	TOF_1I
B. Tournefortii (T161) ♀ ×*B. campestris* (C632) ♂	42	7	0.17	TCF_1I
B. Tournefortii (T161) ♀ ×*S. arvensis* (Ar102) ♂	54	79	1.46	$TArF_1I$
B. Tournefortii (T161) ♀ ×*R. sativus* (R105) ♂	6	2	0.33	TRF_1I

* All the seeds obtained were very small in size, showing their obvious hybrid nature. Thirty of them were sown, all of which proved to be the true hybrids. Therefore, the cross ability was calculated from the total.

** In the F_1 *B. campestris* × *B. adpressa* only 'clasping' is dominant over 'not clasping'.

All of the F_1 hybrids obtained were almost completely sterile, showing deformed stamens whose shrivelled anthers contained almost no functional pollens, except a very few morphologically normal ones which were, presumably, of diad origin.

2. Colchincine-induced tetraploid F_1 hybrids.

Through colchicine treatment of the hybrids the author was successful in obtaining 5 kinds of $4x$-F_1 hybrids and one $2x$-$4x$-chimera plant. They are shown in Table 3 together with their percentage of normal pollens and fertility on selfing as well as on mutual pollination among sister tetraploid hybrids. They were obviously of gigas nature as compared with their corresponding diploid hybrids and showed normal development of stamens whose anthers gave abundant morphologically perfect pollens, save in the case of $4x$-$CAdF_1I$ whose abnormal stamens with deformed anthers gave no pollens like those of their sister diploid hybrids. In most of the young anthers investigated in $2x$-$4x$-TCF_1I concurrence of $2x$- and $4x$-PMCs was noticed. The author was successful in examining the mode of meiosis carried out in both of them. At flowering, there were observed 3 kinds of flowers, viz., those which were the same in morphology as those of the diploid, those showing varying degrees of abnormal development of stamens, and those which were obviously of tetraploid nature with larger petals and normal development of stamens. Though the last kind of flowers were very few in number, the author could determine the percentage of normal pollens under tetraploid condition from them.

3. The result of karyological investigation of the hybrids.

The result of karyological investigation to be reported here is confined only to the mode of chromosome conjugation at MI in the microsporogenesis carried out in the diploid and the tetraploid hybrids, which is shown in Table 4.

TABLE 3. *Colchicine-induced Tetraploid F_1 Hybrids and their Fertility.*

Tetraploid F_1 hybrids	Number of plants obtained	Percentage of normal pollens	Fertility	
			on selfing	on mutual pollination among sister tetraploids
$4x$-$CAdF_1I$	2	—	—	—
$4x$-$CFrF_1II$	3	84	0.00	0.30
$4x$-$FrCF_1II$	7	86	0.00	1.67
$4x$-$FrNiF_1I$	5	87	0.34	—
$4x$-TRF_1I	1	84	0.20	—
$2x$-$4x$-TCF_1I	1	89*	—	—

Note : The figures showing the fertility are the ratio the number of seeds obtained to the number of flowers pollinated. In the case of mutual pollintion the fertility of the plant which showed the highest fertility is given.
* The figure shows the percentage of normal pollen in tetraploid anthers.

TABLE 4. *Mode of Chromosome Conjugation Observed at MI in the Microsporogenesis in the Diploid and the Tetraploid F_1 Hybrids*

Hybrids	Genome constitution	Mode of conjugation	Number of PMCs inves-tigated	Mean frequency appearance of bivalents or qaudrivalents
$CAdF_1I$	AA_d	$(5\text{-}0)_{II}+(7\text{-}17)_{I}$	100	2.28±0.15
$CFrF_1I$	AF	$(7\text{-}0)_{II}+(4\text{-}18)_{I}$	50	3.04±0.12
$CFrF_1II$	〃	〃	168	3.08±0.13
$FrCF_1I$	〃	〃	61	3.10±0.13
$FrCF_1II$	〃	〃	147	2.60±0.13
$FrNiF_1I$	FB	$(7\text{-}0)_{II}+(2\text{-}16)_{I}$	127	3.61±0.18
TCF_1I	TA	$(4\text{-}0)_{II}+(12\text{-}20)_{I}$	106	1.28±0.10
TOF_1I	TC	$(3\text{-}0)_{II}+(13\text{-}19)_{I}$	77	1.45±0.12
$TArF_1I$	TS_{ar}	$(5\text{-}0)_{II}+(9\text{-}19)_{I}$	100	2.48±0.11
TRF_1I	TR	$(7\text{-}0)_{II}+(5\text{-}19)_{I}$	104	3.29±0.19
$4x\text{-}CAdF_1I$	AAA_dA_d	—	—	—
$4x\text{-}CFrF_1II$	$AAFF$	$(7\text{-}0)_{IV}+(4\text{-}18)_{II}$	159	3.36±0.12
$4x\text{-}FrCF_1II$	$AAFF$	〃	121	2.95±0.13
$4x\text{-}FrNiF_1I$	$FFBB$	$(7\text{-}0)_{IV}+(2\text{-}16)_{II}$	126	3.41±0.16
$4x\text{-}TRF_1I$	$TTRR$	$(7\text{-}0)_{IV}+(5\text{-}19)_{II}$	79	3.15±0.22
$2x\text{-}4x\text{-}TCF_1I$	$TA\text{-}TTAA$	$(1\text{-}0)_{IV}+(18\text{-}20)_{II}$	112	0.16±0.03

A. F_1 *B. campestris* × *B. adpressa* ($CAdF_1I$ and $4x\text{-}CAdF_1I$)

The author was barely successful in investigating the meiosis in only one of the 33 $CAdF_1I$ plants, the remaining 32 performing no PMC division due to degeneration of PMCs some time after their differentiation. The phenomenon might perhaps be due to an interaction between *adpressa*-cytoplasm and *campestris*-genes. The question is reserved for future study with the reciprocal hybrid.

In only 16 per cent of the PMCs investigated 17 univalents scattering throughout the spindle area were noticed, in all the others being shown varying number of loosely paired bivalents whose maximum number was 5. Degeneration of PMCs occurred also in the 2 tetraploid hybrids $4x\text{-}CAdF_1I$ and the author could not examine their meiotic division.

B. F_1 *B. campestris* × *B. fruticulosa* ($CFrF_1$, $FrCF_1$, $4x\text{-}CFrF_1$ and $4x\text{-}FrCF_1$)

In all of the 4 different strains of the diploid hybrids rather loosely paired bivalents ranging in number from nil to 7 were confirmed. Detailed investigation in the two strains, $CFrF_1II$ and $FrCF_1II$, however, revealed that the mean of the frequency appearance of bivalents was significantly different between them (Table 5). The former was from the cross C333×Fr103 and the latter from Fr104 ×C333. They showed a remarkable difference in morphology due to the different strains of *B. fruticulosa* concerned as one of the parents. To the author's unexpectation, occurrence of formation of indefinite number of quadrivalents whose maximum number was 7 was observed in their corresponding tetraploid hybrids, $4x\text{-}CFrF_1II$ and $4x\text{-}FrCF_1II$, respectively. Here again was shown a significant difference between them in the mean of the number of quadrivalents (Table 5).

TABLE 5. *Test of Significance of Difference among the Means of the Number of Bivalents and of Quadrivalents in the Diploid and the Tetraploid F_1 Hybrids between B. campestris and B. fruticulosa.*

$FrCF_1II$ bivalents: $\bar{x}=2.60\pm0.13$	$CFrF_1II$ bivalents $\bar{x}=3.08\pm0.13$	$t=2.53$, p: 0.01-0.03
$4x$-$FrCF_1II$ quadrivalents $\bar{x}=2.95\pm0.13$	$4x$-$CFrF_1II$ quadrivalents: $\bar{x}=3.36\pm0.12$	$t=2.16$, p: 0.02-0.05
$t=1.26$, p: 0.2-0.3	$t=1.33$, p: 0.1-0.2	

C. F_1 *B. fruticulosa* × *B. nigra* ($FrNiF_1$ and $4x$-$FrNiF_1$).

The expected configuration 16_I was rarely observed in $FrNiF_1I$, being in only 2 out of 127 PMCs examined. Occurrence of bivalent formation ranging in number from 1 to 7 was recognized in the other cells. In the tetraploid hybrids, $4x$-$FrNiF_1I$, nuclear plates formed exclusively by bivalents (16_{II}) were rarely met with, but in majority of cases they accompanied indefinite number of quadrivalents whose maximum number was 7.

D. F_1 *B. Tournefortii* × *B. campestris* (TCF_1 and $2x$-$4x$-TCF_1).

The mode of chromosome pairing observed in TCF_1I corresponded with that reported by Fukushima and Iwasa (1) in their F_1. *B. Tournefortii* × *B. trilocularis*, of which the MI configuration being represented as $(4-0)_{II}+(12-20)_I$. As stated elsewhere in this report *B. trilocularis* is the synonym of *B. campestris* var. *sarson*. In the single chimera plant, $2x$-$4x$-TCF_1I, the author was able to observe meiosis in relatively many tetraploid PMCs. About 85 per cent of them showed regular nuclear plates with 20_{II}, but the remaining ones presented formation of one quadrivalent.

E. F_1 *B. Tournefortii* × *B. oleracea* (TOF_1).

Nineteen univalents scattering throughout the spindle area were noticed in about a quarter of the PMCs investigated. In the remaining ones appearance of varying number of bivalents was observed, whose maximum number was 3.

F. F_1 *B. Tournefortii* × *S. arvensis* ($TArF_1$).

In 16 of the 100 PMCs investigated 19 univalents taking their position at random above and below the equatorial region were observed, while in the remaining ones occurrence of indefinite number of bivalents was noticed, which ranged in number from 1 to 5.

G. F_1 *B. Tournefortii* × *R. sativus* (TRF_1 and $4x$-TRF_1).

In overwhelming majority of the PMCs investigated formation of varying number of loosely paired bivalents ranging from 1 to 7 was recognized. The PMCs with exclusively univalents (19_I) was only about 9 per cent of the total. In the

tetraploid hybrids, $4x$-TRF$_1$I, nuclear plates with exclusively bivalents (19_{II}) were observed in only about 10 per cent of the total 79 PMCs examined, the others showing formation of varying number of quadrivalents whose maximum number was 7.

Discussion

1. Partially homologous relation among the genomes used.

As seen from the result shown in Table 4 there always occurs a formation of indefinite number of bivalents at MI in the microsporogenesis carried out in all the species and genus hybrids obtained. Whether the bivalents are due to allosyndesis between the two different genomes concerned or to autosyndesis in each of them can be determined in the following way.

Supposing that there occurs autosyndesis in the genome A_d of *B. adpressa* (n=7), the number of autosyndetic bivalents can not exceed 3. On the other hand, the possibility of occurrence of autosyndetic bivalent in the genome A of *B. campestris* is only one as reproted already by the author and other workers (1, 4, 5). Therefore, even if we assume that all such autosyndetic bivalents appear in CAdF$_1$ I, the remaining one (=5–4) can be attributed to allosyndesis between A and A_d.

Similarly, the maximum number of autosyndetic bivalents, if occurring in the genome F of *B. fruticulosa* (n=8), should be 4. Therefore, the possible maximum number of autosyndetic bivalents in CFrF$_1$ and FrCF$_1$ can not exceed 5, *i.e.*, 4 in F and 1 in A. Hence, at least 2 of the 7 bivalents formed in the hybrids must be of allosyndetic origin between the two genomes A and F. However, we can determine more exactly the origin of the 7 bivalents at issue, if we take the quadrivalent formation in the tetraploid hybrids into consideration. The maximum number of quadrivalents formed in $4x$-CFrF$_1$ and $4x$-FrCF$_1$ is 7, corresponding exactly with that of bivalents in the diploid hybrids. Since no quadrivalent formation occurs in either of the parental species under diploid condition, the 7 quadrivalents in the tetraploid hybrids are no dobut the product of auto-alloysndesis between A and F. We can, therefore, say safely that all of the 7 bivalents appearing in the diploid hybrids are of allosyndetic origin.

The same reasoning is applicable in the cases of the F$_1$ *B. fruticulosa* × *B. nigra* (*B*) and the F$_1$ *B. Tournefortii* (*T*) × *R. sativus* (*R*), where the tetraploid hybrids show the same maximum number of quadrivalents as that of bivalents in their corresponding diploid hybrids. Thus we have been able to confirm the occurrence of each 7 allosyndetic bivalents between F and B and T and R.

The number of quadrivalents confirmed in the tetraploid PMCs in the $2x$-$4x$-TCF$_1$I, the artificial chimera F$_1$ *B. Tournefortii* × *B. campestris* var. *sarson*, is only one, being far less than the maximum number of bivalents (=4) in the diploid hybrid. Yet the fact is enough to substantiate the occurrence of allosyndesis between the two genomes T and A. Taking the possibility of occurrence of each one autosyndesis in each of the genomes, we can say that 2 of the 4 bivalents in the

diploid hybrids are of allosyndetic origin. Hybrids between *B. Tournefortii* and other varieties of campestris than *sarson* have been reported (1), in which the maximum number of bivalents formed reaches up to 5.

As already reported by some workers including the author (4, 6, 7, 8) the possibility of the occurrence of autosyndesis in the genome *C* of *B. oleracea* is only one. Accordingly, the possibility of the appearance of 2 autosyndetic bivalents can not be denied theoretically in the F_1 *B. Tournefortii* × *B. oleracea*. However, granted the occurrence of such autosyndetic bivalents, one of the 3 bivalents found in the hybrid is attributable to allosyndesis between the two genome *T* and *C*.

Three of the 5 bivalents formed in the F_1 *B. Tournefrotii* × *S. arvensis* (S_{ar}) are obviously due to allosyndesis between *T* and S_{ar}, because the number of autosyndesis theoretically expected in the genome S_{ar} is only one as reported by the author (4, 8).

From the facts confirmed above it is concluded that there exists a partially homologous relation between A_d—*A*, *F*—*A*, *F*—*B*, *T*—*A*, *T*—S_{ar}, and *T*—*R*.

Since the affirmation of the existence of the 3 different primary genomes, *A*, *B*, and *C*, by Morinaga (9) and U(11) no other primary genomes have been reported in *Brassica*, save *T* of *B. Tournefortii* (1, 11). Though the two new genomes, A_d and *F*, have been found in the present study, such genomes as those with 11 or 12 chromosomes found in *B. elongata* (n=11), *B. monensis* (n=12) and *B. Wrightii* (n=12) still remain untouched, whose relationship to the known genomes will be made clear in future experiments. The genome A_d is not only the lowest in chromosome number of all the genomes in *Brassica* but also of those found in the tribe *Brassiceae*, the same number being noticed only in those of the two species, *Diplotaxis erucoides* and *Conringia orientalis*. The genome *F* with 8 chromosomes is distinctly different from *B* and shows a remarkably high affinity to both *B* and *A*. The two genomes will, no dobut, furnish us with important informations for the elucidation of the genome differentiation in *Brassica* and allied genera.

It is a remarkable fact that, in spite of the possibility of the occurrence of each one autosyndesis in the genomes *A*, *B*, *T* and *R*, no autosyndetic bivalents have actually been confirmed in the hybrids whose genome constitutions are *FA*, *FB* and *TR*. The author has pointed out previously that, although even the slightest possibility of occurrence of autosyndesis in the used genomes, *A*, *B*, *C*, S_{ar} and *R*, can not be neglected in determining the origin of bivalents formed in the hybrids, the occurrence of such a bivalent in each of the genomes seems to be only occasional and might virtually be out of the question (4, 8). The fact above-stated obviously illsutrates the author's reference.

It should be mentioned that the maximum number of frequency appearance of allosyndetic bivalents between any two genomes shows only the upper variation limit of conjugation and does not necessarily represent the actual number of partially homologous chromosomes existing in the genomes. The same is applicable

to the case of autosyndesis, especially in such genomes of *Brassica* and allied genera being proved to be of aneuploid nature derived from a common original genome (4, 8). Rajan and Hardas (12) observed in their artificial as well as spontaneous triploid plants of *Eruca sativa* (*E*, n=11) that the sum of the numbers of trivalents and bivalents frequently exceeded 11 and sometimes amounted up to 16. They concluded from the fact that the maximum number of autosyndesis in the genome *E* was 5. On the other hand, U, Nagamatsu and Mizushima (13) reported that the maximum number of bivalents formed in their *EC* hybrid, the F_1 *E. sativa* × *B. oleracea*, never exceeded 3. The origin of these 3 bivalents is ambiguous and can not be attributed only to autosyndesis in *E*, because there are the possibilities of one autosyndesis in *C* and also allosyndesis between *E* and *C*. The situation is rather complex and can not be explained readily. One possible explanation is that in such a species as *E. sativa* being widely distributed throughout the vast area from the Mediterranean to the Middle Eastern region there have occurred many an ecotype or even an ecospecies which are considered naturally to be various in autosyndetic capacity. Another explanation is that the autosyndetic capacity under triploid condition may be modified under haploid condition with the presence of a partially homologous genome. If the second explanation is applicable, the fact stated above is a good illustration showing that the maximum number of frequency appearance of autosyndetic pairs does not necessarily represent the actual number of chromosomes capable of pairing within a genome.

The significant difference in the mean of the number of allosyndetic pairs in the two F_1 hybrids, $CFrF_1II$ and $FrCF_1II$, is likely to show the difference in differentiation between the two *F* genomes involved, as in the case between *B. campestris* var. *sarson* (=*B. trilocularis*) and other *campestris* varieties. A definite answer for the problem will be given in a future report.

2. Genomic inter-relationship in *Brassiceae* so far studied.

Putting together the facts confirmed in the present experiment with those already reported (4, 8), we can show the genomic inter-realtionship so far studied in the tribe *Brassiceae* in a summarized, diagrammatical representation as seen in Fig. 1. In making a general survey of Fig. 1 one will notice a remarkable fact that the degree of affinity among the genomes represented in terms of the number of allosyndesis is not always higher within the genus *Brassica* than between *Brassica* and other genera. For instance, the probable maximum number of allosyndetic bivalents that appeared, *i.e.*, the sum of the large- and small-sized figures, is 3 between *A*—*B*, 4 between *B*—*C*, and 3 between *C*—*T*, whereas it is 7 between *T*—*R*, 8 between *B*—S_{ar}, and 8 between *A*—*E*. Even if we take the possibility of autosyndesis in each of the genomes into consideration, a similar situation is observable among the genomes *A*—*T*—*R* and *A*—*B*—S_{ar}. Thus the morphological calssification at genus level is not parallel to the phylognetical relationship, rendering it difficult to

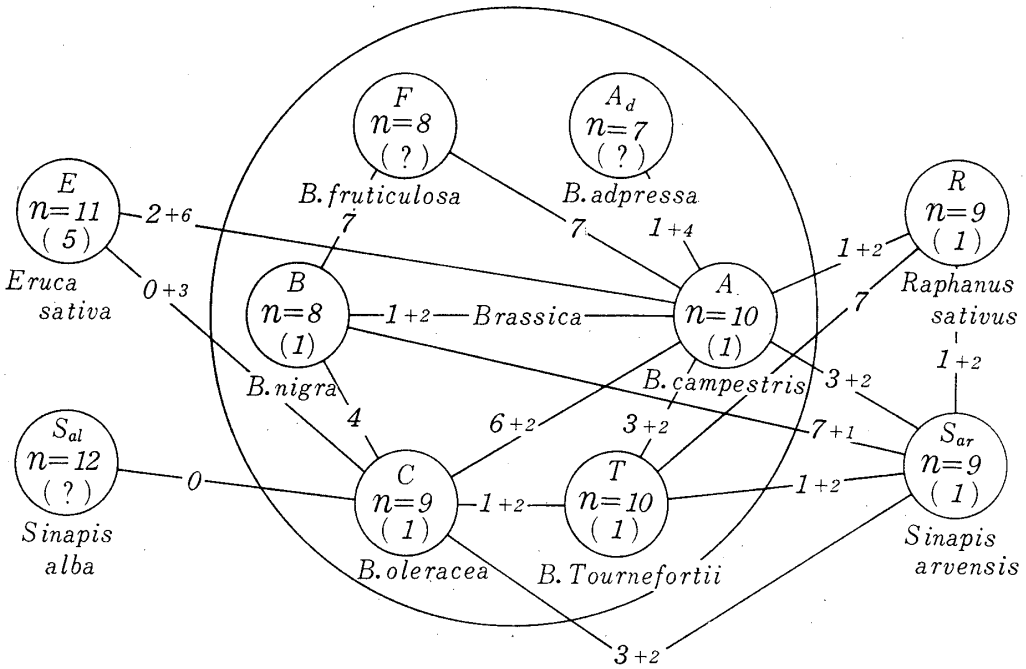

FIG. 1. Genomic inter-relationship in *Brassiceae* represented by the amount of allosyndesis.

Numbers within the circle enclosed in parentheses denote the maximum autosyndetic pairing within that genome. Numbers connecting the genomes show the probable number of alloysndetic pairing that occurred. Large-sized numbers show the lower limit of allosyndetic paris. The sum of the large- and small-sized numbers gives the upper limit.

Note: The symbol *S* which was used previously to represent the geome of *S. arvensis* is described here as S_{ar} to distinguish it from that of *S. alba* (S_{al}). From the result of Rajan and Hardas (12) the maximum number of atuosyndesis within the genome *E* has been altered, for rigorousness' sake, from 3 to 5.

The *T*—*A* relation has been cited from the result of other workers (1), because the author's observation was confined only to that between *T*—*A'* (the genome of *B. campestris* var. *sarson*).

fix generic boundaries among the species used from the latter point of view. As has been stated in the previous papers (4, 8), it is obvious from their continuous chromosome numbers and partially homologous relations that all the genomes at issue are of aneuploid nature having been derived from a common original one. They still retain their chromosome structure to such an extent as to cause attraction between homolgous parts. In this sense they can be said to be nearly on the same level of differentiation.

3. Artificial allopolyploids in *Brassiceae.*

The origin and the kinds of artificial allopolyploids obtained so far by the author in *Brassiceae* are shown diagrammatically in Fig. 2.

It has been stated already by the author (4, 14) that the synthetic digenomic tetraploids show normal or nearly normal meiosis, regardless of the occurrence of

quadrivalent formation in some of them, whereas the di- and the trigenomic hexaploids show a remarkably irregular mode of division with indefinite number of polyvalents and univalents. From this fact he has pointed out that in *Brassica* and allied genera any higher allopolyploidy than digenomic tetraploidy seems to be difficult to breed true. Iwasa (15) made a follow up examination of the meiotic behavior in numerically eu-hexaploid offsprings obtained by successive selfing of his *AABBCC* hexaploid F_1 hybrids between *B. carinata* (*BBCC*) and *B. pekinensis* (*B. campestris* var. *pekinensis*, *AA*) from F_2 up to F_5. He observed nearly the same meiotic irregularity in the F_1 hybrids as that in the author's *AABBCC* hexaploid and noticed an increase of frequency appearance of univalents with the corresponding decrease in the number of normal MII nuclear plates in the progeny as the generation proceeded, leading to the augmentation in the number of aneuploid offsprings towards later generations. He also noticed that the hexaploids were easily fertilized by pollens of *B. cernua* (*AABB*) under uncontrolled pollination, resulting in the offsprings, presumably, of the genome constitution *AABBC* which seemed easy to collapse into descendants with the tetraploid geome constitution *AABB*. Takeda* has engaged in an extensive study of progenies of *AABBCC*

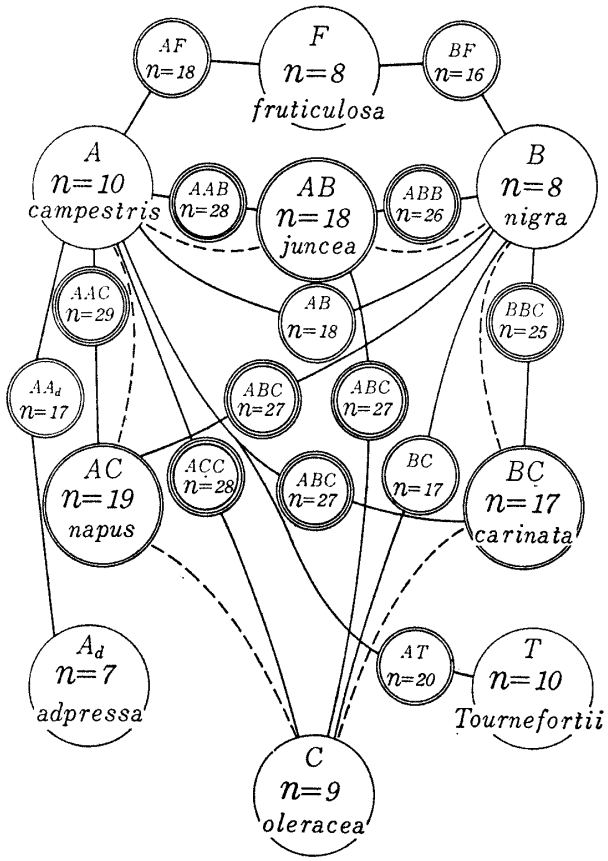

FIG 2. (a)

* Takeda (unpublished).

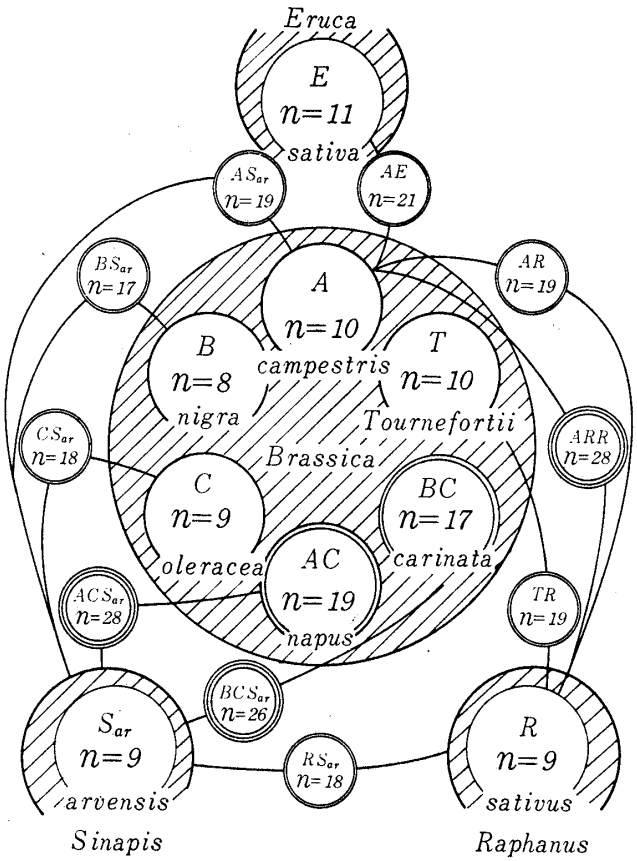

FIG 2. (b)

FIG. 2. Diagrammatical representation of origin and kinds of the synthetic plants obtained by the author in *Brassiceae*.
(a) Those obtained within *Brassica*.
(b) Those obtained between *Brassica* and allied genera.
All the plants are represented by their haploid genome constitution. Large circles with species name show the spontaneous species. Single, double and triple circles denote diploid, tetraploid and hexaploid genome constitutions, respectively. Broken lines in the figure (a) show the process of spontaneous establishment of the digenomic species in *Brassica*.

hexaploids obtained by the author and his collaborators from the 4 different crosses, $(B\times C)\times A$, $BC\times A$, $AC\times B$ and $AB\times C$. The results so far obtained by him show the general tendency towards the break-down of the original hexaploid genome constitution in the progenies as the generation proceeds, though the way of shift is somewhat different in different progenies. The facts above-mentioned seem to show that the main reason for the occurrence of no spontaneous higher allopolyploidy within and among the genera at issue is due to meiotic irregularity which result in the break-down of the original genome constitution.

Most of the author's synthetic plants have been made for purely karyogenetical purpose and are of little practical value. However, a few of them obtained from such crosses as turnip (AA) $\times$ radish (RR) and Chinese cabbage (AA) $\times$ common

cabbage (*CC*) suggested that the artificial synthesis was promising as a method of breeding new root crops and leaf vegetables, if a variety with desirable characteristics was chosen and the synthesis was done at the tetraploid level (16). It is needless to say that, even if a synthetic plant is of no practical value as such, it can be used as an important material for introducing necessary genes from one genome to the other, for making an alien substitution race, and for securing an alloplasmatic strain, as seen in the breeding of the potato (17, 18 ,19, 20, 21, 22), the tobacco (23, 24), and other plants.

Hosoda (25, 26) isolated several *AACC* tetraploid strains after successive selfing of the author's *AACCCC* hexaploid between Chinese cabbage and common cabbage. Some of these strains are now cultivated widely throughout Japan as a desirable green fodder because of their leafiness and winter hardiness. The former character is obviously from the *A* genome and the latter is, presumably, from the *C* genome. Hosoda's *AACC* strains (or 'synthetic *napus*' as they are called popularly in the market) have been used as a breeding material for introducing disease resistance from *C* into *A*. Hiratsuka No. 1, a new Chinese cabbage variety resistant to bacterial soft rot, was obtained as a result of repeated backcrossing of a Chinese cabbage strain with the 'synthetic *napus*' (27). Mizushima and Katsuo (28) obtained several common cabbage strains having cytoplasm of *B. nigra*, taking a synthetic *B. carinata* with *nigra*-cytoplasm as the non-recurrent parent and pollinating it with the same pollens of a self-incompatible common cabbage plant which was continued vegetatively. These alloplasmatic common cabbage strains proved to be practically self-compatible, if not completely, and to breed true by selfing without any loss of vigor. When taken as the female plants, they cross easily with the recurrent cabbage parent, producing abundant seeds which gave rise to practically self-compatible plants. However, when taken as the male plants, they are utterly incompatible with the recurrent parent, giving only occasional seeds which developed into exclusively self-incompatible offsprings. The fact shows obviously that suppression to a high degree of the effect of incompatibility genes contained in the *C* genome of the recurrent parent is brought about by the action of *nigra*-cytoplasm. Therefore, it has been pointed out by the author (29) that substitution of nucleus can be a new technique applicable to the breeding of the crops belonging to the cabbage group. Monogenomic plants having cytoplasm of a spontaneous digenomic species were reported in *Brassica*. Iwasa (30, 31, 32) observed in his *carinata*-cytoplasmic *AA*-plants (*B. campestris* var. *pekinensis*) several alloplasmatic effect manifested in the shape of leaves, in the size of corolla, in the content of chlorophyll, and etc., as compared with the recurrent, spontaneous *AA*-plants, though there was no noticeable effect on the incompatibility of the *A* genome used.

Since the early 1950's studies on breeding by means of artifiical synthesis in *Brassica* have been common in Japan and northern Europe, especially in Sweden.

The workers in this field of study have dealt mainly with synthetic *B. napus* (*AACC*) for the examination of the possibility of obtaining new productive forms of rape (33, 34, 35), swede (34, 35, 36, 37, 38), fodder rape (39, 40, 41, 42, 43) and leaf vegetable (44), though some di- and trigenomic hexaploids, synthetic *B. carinata* and *B. juncea* were examined as to their practicableness in the earlier period (45, 46). In Japan the study seems to have been accelerated by the development of an embryoculture technique which enables the workers to obtain hybrids from difficult species crosses rather easily (47, 48). A few of the synthetic rape strains obtained between turnip rape and kale reported by Olsson (35) proved to outyield a dominating Swedish rape variety, showing satisfactory oil content and better winter hardiness, or to be comparable in yield to the latter but with higher oil content and better winter hardiness. One strain even proved to be a productive fodder rape which outyielded the best introduced fodder rape from England, being higher in protein content and lower in crude fibre content. As to the synthetic swede between turnip and kale he remarked that, though the strains so far obtained yielded less than the best marketed variety, they showed wider variation in root shape than spontaneous varieties and one strain was promising to give fairly satisfactory materials by selection. He added that the artificial swede strains were mainly used for crosses with spontaneous strains and these crosses resulted in the production of valuable breeding materials. Namai and Hosoda (38) also observed in their synthetic swede strains between turnip and kohlrabi a wider variation in root shape than spontaneous strains together with some distinct characters. They laid stress on the crosses between the artificial and spontaneous materials to obtain excellent semi-synthetic strains for practical use. Sarashima (43) observed a remarkable heterosis manifested in the yield of the hybrids between his synthetic fodder rape and some commercial rape varieties and recommended the utilization of their progeny for practical use.

The general situation observed so far shows that synthetic plants obtained even between excellent commercial varieties can not, in most cases, readily be used practically as such, because, in spite of their practically valuable advances for some distinct characters, they are usually not equal to the best spontaneous varieties in all agronomic characters. They are valuable, as has been pointed out by Olsson (35), because of their possibility of being utilized in breeding not only for their wider variation in characters existing in spontaneous amphidiploids but also for the variation existing in the primary species.

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References

1) Fukushima, E. and Iwasa, S., *J. Fac. Agr. Kyushu Univ.*, **13**, 743 (1966)
2) Mizushima, U. and Tsunoda, S., *Tohoku J. Agr. Res.*, **17**, 250 (1967)
3) Olsson, G., *Hereditas*, **40**, 398 (1954)
4) Mizushima, U., "*Karyogenetic Studies on Brassiceae*", Gihodo, Tokyo (1952) (in Japanese)
5) Ramanujam, S., *Proc. Indian Acad. Sci.*, **14**, 25 (1941)
6) Howard, H.W., *J. Gen.*, **36**, 239 (1938)
7) Fukushima, E., *J. Dept. Agr. Kyushu Univ.*, **7**, 281 (1945)
8) Mizushima, U., *Tohoku J. Agr. Res.*, **1**, 1 (1950)
9) Morinaga, T., *Cytologia*, **6**, 62 (1934)
10) U,N., *Jap. J. Bot.*, **7**, 389 (1935)
11) Sikka, S.M., *J. Gen.*, **40**, 441 (1940)
12) Rajan, S.S. and Hardas, M.W., *Ind. J. Gen. Pt. Breed.*, **24**, 15 (1964)
13) U, N., Nagamatsu, T. and Mizushima, U., *Cytologia*, Fujii Jub. Vol., 437 (1937)
14) Mizushima, U., *Tohoku J. Agr. Res.*, **1**, 15 (1950)
15) Iwasa, S., *J. Fac. Agr. Kyushu Univ.*, **13**, 309 (1964)
16) Mizushima, U., *Kagaku*, **20**, 399 (1950) (in Japanese)
17) Lam, R., *Hereditas.*, **31**, 1 (1945)
18) Black, W., *Ann. Appl. Bot.*, **34**, 631 (1947)
19) Swaminathan, M.S., *Züchter*, **20**, 358 (1950)
20) Swaminathan, M.S., *Amer. Potato J.*, **28**, 472 (1951)
21) Taguchi, K. and Irikura, Y., *Jap. J. Breed.*, **9**, 66 (1959) (in Japanese with English summary)
22) Kawakami, K. and Matsubayashi, M., *Jap. J. Breed.*, **10**, 119 (1960) (in Japanese with English summary)
23) Clayton, E.E., *Phytopathology*, **38**, 5 (1948)
24) Clayton, E.E. and McMurtrey, J.E., *Fmrs' Bull. U.S. Dept. Agr.*, **2032**, 70 (1950)
25) Hosoda, T., *Ikushukenkyu*, **4**, 91 (1950) (in Japanese with English summary)
26) Hosoda, T., *Jap. J. Breed.*, **3**, 44 (1953) (in Japanese with English summary)
27) Shimizu, S., Kanazawa, K. and Kobayashi, T., *Bull. Hort. Res. Sta., Ser. A*, **157** (1962)
28) Mizushima, U. and Katsuo, K., *Proc. X Int. Congr. Gen. II*, 191 (1958)
29) Mizushima, U., *Recent Advances in Breeding*, **2**, 44 (1961) (in Japanese)
30) Iwasa, S., *J. Fac. Agr. Kyushu Univ.*, **12**, 201 (1963)
31) Iwasa, S., J. *Fac. Agr. Kyushu Univ.*, **12**, 213 (1963)
32) Iwasa, S., J. *Fac. Agr. Kyushu Univ.*, **12**, 229 (1963)
33) Hoffmann, W., and Peters, R., *Züchter*, **28**, 40 (1958)
34) Olsson, G., *Hereditas*, **46**, 351 (1960)
35) Olsson, G., "*Recent Plant Breeding Research, Svalöf* 1946–1961", 179, Almqvist & Wiskel, Stockholm (1963)
36) Olsson, G., Josefsson, A., Hagberg, A. and Ellerström, S., *Hereditas*, **41**, 241 (1955)
37) Hosoda, T., Namai, H. and Goto, J., *Jap. J. Breed.*, **13**, 99 (1963) (in Japanese with English summary)
38) Namai, H. and Hosoda, T., *Jap. J. Breed.*, **17**, 194 (1967) (in Japanese with English summary)
39) Hosoda, T., *Mem. Fac. Agr. Tokyo Univ. of Education*, **7**, 1 (1961) (in Japanese with English summary)

40) Sarashima, M., *Jap. J. Breed.*, **14**, 226 (1964) (in Japanese with English summary)
41) Sarashima, M., *Jap. J. Breed.*, **15**, 245 (1965) (in Japanese with English summary)
42) Sarashima, M., *Jap. J. Breed.*, **16**, 158 (1966) (in Japanese with English summary)
43) Sarashima, M., *Jap. J. Breed.*, **17**, 26 (1967) (in Japanese with English summary)
44) Shinohara, S. and Kanno, M., *Nogyo oyobi Engei*, **36**, 1189 (1961) (in Japanese)
45) Olsson, G., *Hereditas*, **46**, 171 (1960)
46) Frandsen, K.J., *Dansk. Bot. Akkiv.*, **12**, 1 (1947)
47) Nishi, S., Kawata, J. and Toda, M., *Jap. J. Breed.*, **8**, 215 (1959) (in Japanese with English summary)
48) Nishi, S., Kawata, J. and Toda, M., *Bull. Hort. Res. Sta.*, Ser. A, III (1962) (in Japanese with English summary)